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14. ABSTRACT

During the reported period, we have been able to generate all experimental groups of mice on E3 and E4 genetic background and have completed the experiments with two age groups: 3 and 9 months. Three behavioral paradigms were used to evaluate the differences in cognitive performance between the genotypes and age groups. The analysis of the results (two parameters in Morris Water Maze) demonstrated that while the young mice (sham and injured) of both genotypes recover faster, the differences (do not reach significance. In the other two paradigm – Elevated Plus Maze and Contextual Fear Conditioning) there were differences in performance between the genotypes in the old groups only. All brain samples from mice of the above groups have been processed and libraries generated for mRNA-seq. All libraries have been sequenced and the sequencing files transferred from NGS Center at UPenn, processed and analyzed. Differential expression tables showed clearly delineated GO categories of up- and down-regulated genes between sham and injured mice. The results of the study at this stage have been presented at the SfN meeting in Chicago in October, 2015.

15. SUBJECT TERMS

Abca1 global deletion, APOE targeted replacement, complex breeding, CCI model optimization, mRNA library generation, high throughput massive parallel sequencing, analysis

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Contents

1 Introduction

Patients carrying apolipoprotein E4 (APOE) ?4 allele are more susceptible to poor neurological outcome after traumatic brain injury (TBI). Furthermore, the inheritance of APOe4 is the only proven genetic risk factor for sporadic Alzheimer disease (AD). Importantly, TBI is a risk factor for the subsequent development of AD particularly among APOE?4 carriers. ATP binding cassette transporter A1 (ABCA1) is a lipid transporter that controls the generation of HDL in plasma and ApoE-containing lipoproteins in the brain which are important for repairing axonal damage after TBI. We demonstrated that lack of Abca1 increases amyloid plaques and decreased APOE protein level in AD-model mice. In this proposal we will test the hypothesis that ABCA1 differentially affects the response of mice expressing human APOE isoforms to TBI. We will evaluate the immediate as well as the long-term effects following brain injury. The Aims are: 1) To determine the effect of Abca1 deficiency on the response to TBI in mice expressing human APOE3 and E4 isoforms, and 2) 2. To evaluate the development of AD-like phenotype in APP expressing mice exposed to TBI and to determine if the differences in the phenotype are mediated through ABCA1.

2 Keywords

Traumatic brain injury, APOE isoforms, ABCA1, Alzheimer disease, APP mice, amyloid beta, axonal injury, inflammatory reaction, trnascriptome, high-throughput massive parallel sequencing, mRNA-seq, behavioral testing, memory impairement, recovery.

3 Overall Project Summary

During the reported period, we have been able to generate all experimental groups of mice on E3 and E4 genetic background and have completed the experiments with two age groups: 3 and 9 months. Three behavioral paradigms were used to evaluate the differences in cognitive performance between the genotypes and age groups. The analysis of the results (two parameters in Morris Water Maze) demonstrated that while the young mice (sham and injured) of both genotypes recover faster, the differences (do not reach significance. In the other two paradigm – Elevated Plus Maze and Contextual Fear Conditioning)there were differences in performance between the genotypes in the old groups only. All brain samples from mice of the above groups have been processed and libraries generated for mRNA-seq. All libraries have been sequenced and the sequencing files transferred from NGS Center at UPenn, processed and analyzed. Differential expression tables showed clearly delineated GO categories of up- and down-regulated genes between sham and injured mice. The results of the study at this stage have been presented at the Soc. For Neuroscience meeting in Chicago in October.

3.1 Cognitive performance

3.1.1 Changes in anxiety related behavior following TBI

A) Progress already reported

On day 4 following injury, we tested all mice in the EPM for anxiety-related behaviors. Mice were excluded based on low total exploration of the maze, indicating higher motor coordination problems. Injured mice from both genotypes and age groups showed an increased amount of time spent in the open-arms of the EPM (Figure 1), however only the data from the old E3 and E4 mice were significant ($p<0.001$, n=7-9). Among the old mice, TBI causes a significant change in anxiety behavior in E4 mice, as seen by the increase in percent time spent in open arms ($p<0.001$), whereas the difference between E3 sham and injured was not significant. Additionally, the percent time spent in open arms by the injured E4 mice was significantly higher compared to E3 injured mice ($p<0.05$), possibly suggesting a higher impact of TBI on anxiety in E4 mice. There is a strong trend for TBI to lead to similar decreases in anxiety in younger mice, however, the low group number may be a limitation for significance (n=4-5).

B) Progress after the last Quarterly report

Upon adding more mice to the young groups (Figure 5), TBI causes a significant increase in percent time spent in open-arms in young mice ($p<0.01$; n=6-7), in additional to the old mice ($p<0.005$). Among E4 animals, TBI

caused a significant change in anxiety behavior in both young ($p<0.05$) and old ($p<0.01$). The difference in the old E3 and E4 injured animals remained the same as before.

3.1.2 TBI causes cognitive impairments as measured in MWM performance

A) Progress already reported

During the acquisition trials of MWM (days 7-11), the ability of sham and injured mice to form a spatial relationship between a hidden platform and visual cues was assessed. There was no interaction seen between injury/genotype and training day performance in the young age group (Figure 2), as determined by Two-way Anova, however performance was significantly affected by genotype/injury ($p<0.01$) and training day ($p<0.0001$). Due to small group size, there are limitations to post-hoc analysis, however, there is a strong trend for injury to affect performance only in E3 mice, but not in E4. In older mice (Figure 3), a similar affect is seen, with injury affecting performance only in E3 mice, suggesting that regardless of age, injury does not affect E4 mice performance due to diminished performance prior to injury. Similarly to young mice, there is no interaction between injury/genotype and training day performance in the old mice, but performance was significantly affected by genotype/injury ($p<0.0001$) and training day ($p<0.0001$). The interpretation of these results requires taking into account the difference in the performance between E3 and E4 expressing mice: it has been repeatedly demonstrated that E4 expressing mice perform worse in a number of paradigms which may influence the magnitude of the response to variety of environmental insults, including TBI. In our case it seems that TBI would affect performance of mice with "normal" status brains i.e. those of E3 expressing mice, but less of mice with "injured" status brain as could be classified by E4. Nevertheless, all groups demonstrated an ability to learn, E4 mice including, just at a slower rate.

B) Progress after the last Quarterly report

Upon adding more mice, we do not see a change to the significance in genotype/injury ($p<0.0001$) and training ($p<0.0001$) in both young (Figure 6) and old (Figure 7) animals. However, in the young mice, the E4 sham animals are learning at a faster rate than their TBI counterparts, similar to the E3 animals. This difference is not seen among the old E4 animals, suggesting an age-related affect of TBI on MWM performance, in that as E4 animals get older, their brains convert from "normal" status brains to "injured" status brains, as discussed previously.

3.1.3 TBI causes age-dependent behavioral changes in Contextual Fear Conditioning

A) Progress already reported

Contextual fear conditioning paradigm is able to test the ability to learn the context that triggers fear. On post-injury days 13-14 mice were trained to learn an association between their environment and a foot shock, and then we tested their ability to remember this context. TBI caused an age-dependent effect in the freezing response during this paradigm (Figure 4), in that only the aged E3 mice performed differently compared to E3 sham animals ($p<0.05$). This response to injury was not seen in the younger E3 mice, and there is no effect on the E4 performance regardless of age. However, in the case of the APOE4 mice, similar to the lack of change in MWM performance, no difference could indicate an "injured" brain status prior to injury. This may be indicated by the lower freezing times in E4 sham mice compared to E3 sham ($p<0.05$) in the older age group. The lack of difference between E3 and E4 mice in the young age group may be due to small group size.

B) Progress after the last Quarterly report

Upon adding more mice to the young mice groups (n=6-7)(Figure 8), there are no changes in values of significance or the differences seen among sham and TBI between E3 and E4.

3.2 Differential Gene Expression

3.2.1 TBI leads to genome-wide injury- and genotype-dependent changes of gene expression

A) Progress already reported

We performed high-throughput sequencing of mRNA-seq libraries generated from total RNA isolated from cortices and hippocampi of 4 young males each/each genotype. Quality Controlled and trimmed Fastq files were aligned to the mouse genome - mm9 assembly, using STAR - an ultrafast aligner installed on a 56 core Linux platform with 128 GB of RAM. This computer and a similar one are available, including through secure remote shell connection (SSH), 24/7 to the PI and several lab members trained to analyze ChIP-seq and mRNA-seq data. To identify differentially expressed genes in mice subjected to sham and TBI, BAM (Binary Alignment Map) files generated by STAR were used as input files for edgeR package available through Bioconductor. Peak calling (Feature summarization) from within edgeR was performed using featureCounts, which is part of Rsubread package. The output tables (sham vs TBI) for each of the y-E3 and y-E4 genotypes were further processed in Excel to prepare input files suitable for submission in DAVID and IPA. Lists of differentially expressed genes, with cutoff of $p < 0.05$, were compared for common and l unique genes in each of the genotypes and then analyzed in DAVID to identify GO clusters and categories significantly enriched. In both E3 and E4 young mice, TBI caused an increase in functional annotation categories related to inflammation and immune response (Table 1) while the genes in gene categories related to neuronal structure, neuronal signaling through transport of ions and neurotransmitters and metabolism were down-regulated. We have been able to identify up-regulated genes categories specific only to E3, and those include response to hypoxia and oxygen levels (Table 2). Remarkably, these same categories were among down-regulated in E4 mice post-injury (Table 3). Down-regulated genes in E4 mice clustered in gene categories associated with cell structure and organization. In contrast, in E4 mice gene categories Cell Localization, Cell Migration and Cell Death were up-regulated. All tables with legends are at the end of this file, after the figures.

B) Progress after the last Quarterly report

In taking the common genes among the Young mice and analyzing this list with DAVID, we identified common up-regulated and down-regulated categories (Figure 9), with p-values and Fold enrichment shown for each category. These charts correspond to the values seen in Table 1, with inflammation and immune response up-regulated and neuronal structure and development down-regulated. Additionally, in taking the list of genes with cutoff $p<0.05$ for all groups and analyzing with DAVID, we made heat maps of the Log(p-value) for categories of interest for both upregulated and downregulated genes (Figure 10). These heatmpas correspond to the data seen in the previous figures, however, the old E3 mice are an outlier, with no Bio-function found in approximately half the categories, including expected upregulated categories, such as inflammation and immune response. This suggests a possible issue with the sequencing of this group, and we plan to remake the libraries for new sequencing. The Log(p-value) of the individual genes are shown for the young E3 and E4 mice in volcano plots (Figure 11), with certain genes related to microglia-activity pulled out among up-regulated genes and genes related to neuronal development pulled out among the down-regulated genes, showing common trends in the pattern of expression among the young mice, if at different levels of significance for differences in expression between TBI versus sham. Additionally, the differential expression of the reads for the common genes between young E3 and E4 animals are shown in a scatterplot (Figure 11). Again, using the list of common genes between young E3 and E4 animals with cutoff $p<0.05$, the upregulated and downregulated genes were separately analyzed with IPA for upstream effectors and their pathways within these lists (Figure 12). IFNα/β was the highest common upstream effector among the upregulated common gene list and Creb1 was the highest common upstream effector among the downregulated gene list. Genes in each pathway are shown at the differential expression level found in the young E3 mice. Overall, these pathways demonstrate an upregulation of an inflammation pathway corresponding to what we have found overall using RNA-sequencing.

3.3 Actual and Anticipated problems

By the end of the reported period of the award we can outline 2 major problems.

A) The generation of APP/Abca1-ko mice is going very slow, and will not be able to generated the groups until the end of the calendar year.

B) Considering the extremely interesting results in terms of the differential gene expression, an approach to validate the inflammatory condition in the brain would require substantial amount of effort for to do all IHC staining

and microscopic analyses.

4 Key Research Accomplishments

- Behavioral tests for 2 age groups and 2 genotypes have been completed
- For the same groups we have sequencing data and differential gene expression tables with GO analysis.

5 Conclusion

At this stage of the study we have the confidence that we will be able to report highly significant results on the differences between transcriptomes of young and adult APOE expressing mice likely to affect the recovery form TBI and possible therapeutic approaches.

6 Publications, Abstracts, and Presentations

1. **Peer-Reviewed Scientific Journals:**

   Lefterov, I., et al. (2015). "RNA-sequencing reveals transcriptional up-regulation of Trem2 in response to bexarotene treatment." **Neurobiol Dis 82: 132-140.**

   Mounier, A., et al. (2015). "Bexarotene-Activated Retinoid X Receptors Regulate Neuronal Differentiation and Dendritic Complexity." **Journal of Neuroscience 35(34): 11862-11876.**

   Fitz, N. F., et al. (2015). "Opposing effects of Apoe/Apoa1 double deletion on amyloid-b pathology and cognitive performance in APP mice." **Brain 138(12); December Issue).**

2. **Poster Presentations:**

   Castranio, E.L., et al. (2015). "Effect of Age and APOE Isoform on Traumatic Brain Injury in Mice." **Society for Neuroscience Annual Meeting, 2015, Chicago.**

7 Inventions, Patents and Licenses

NA

8 Reportable Outcomes

NA

9 Other Achievements

NA

10 References

NA

11 Figures

All Figures and Tables follow on the next pages.

Figures

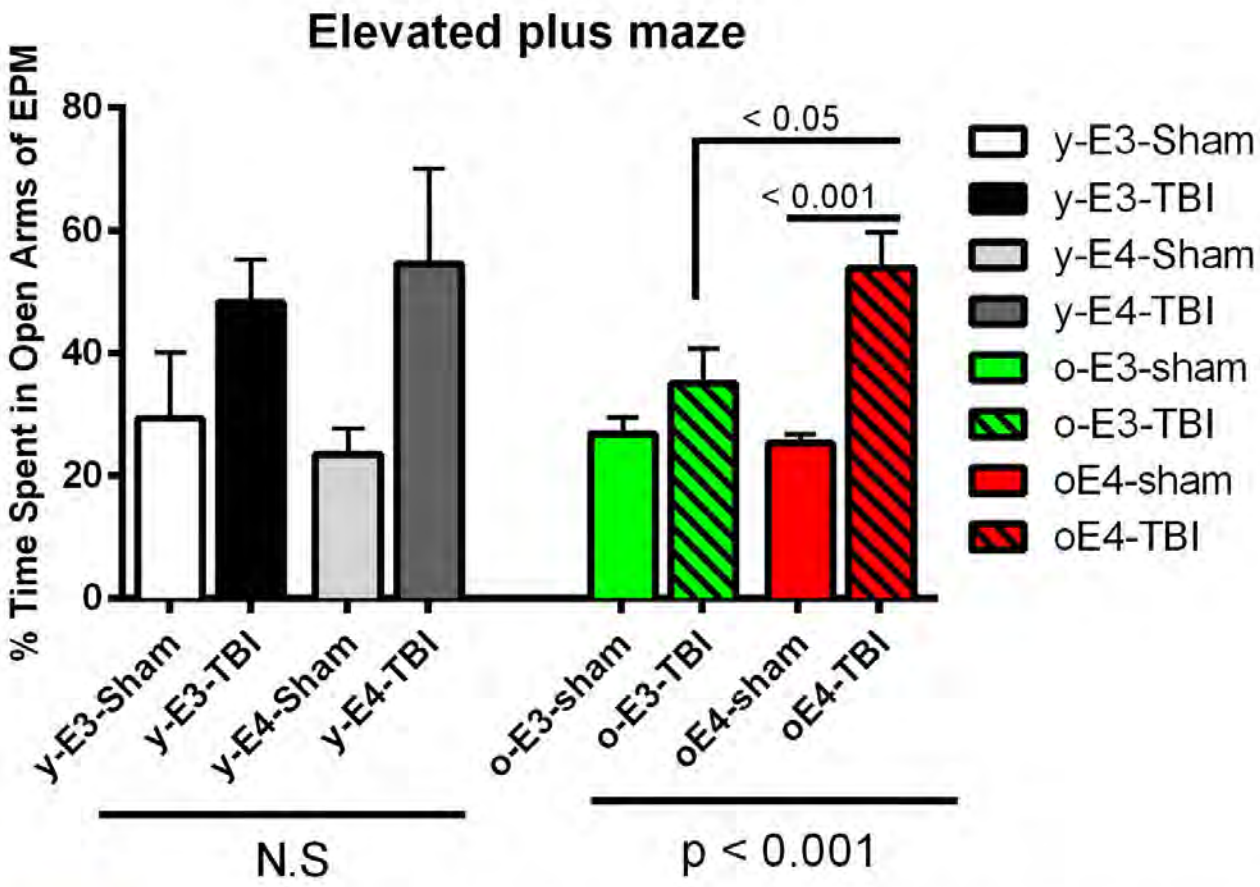
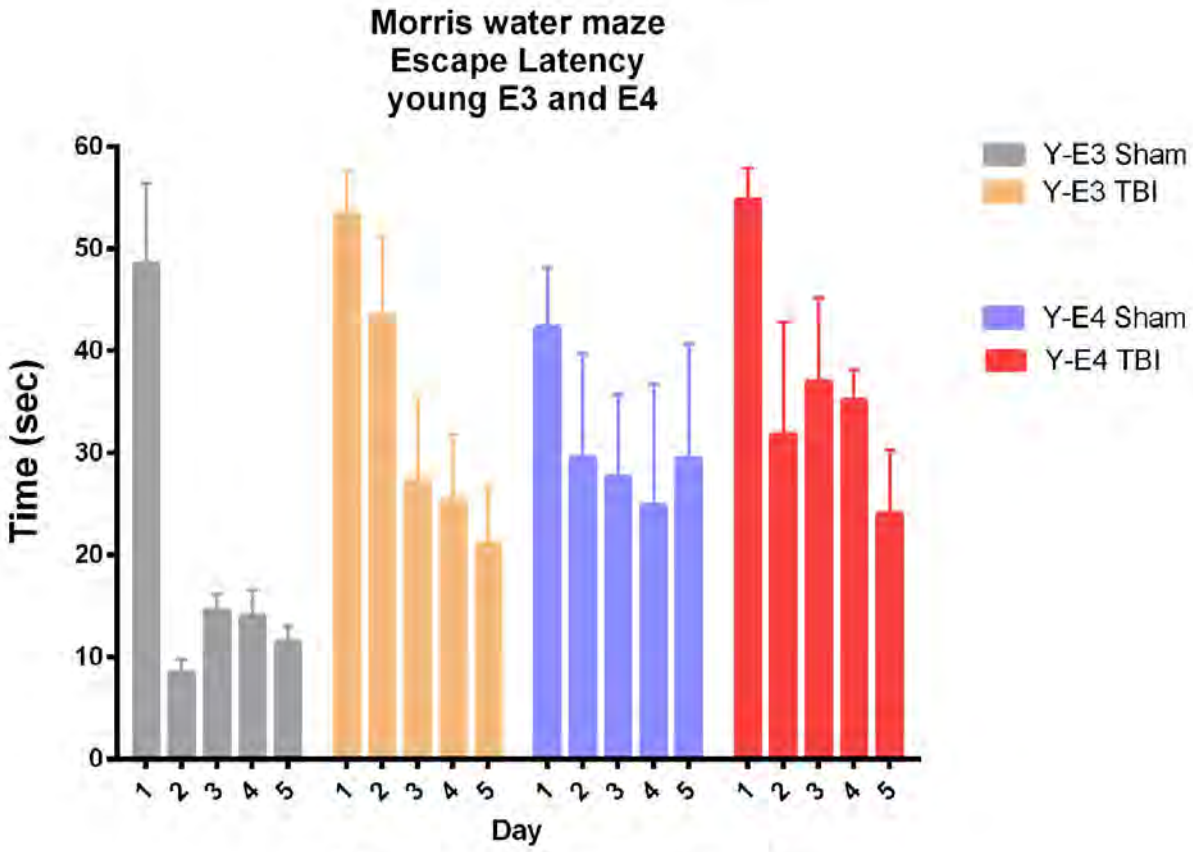
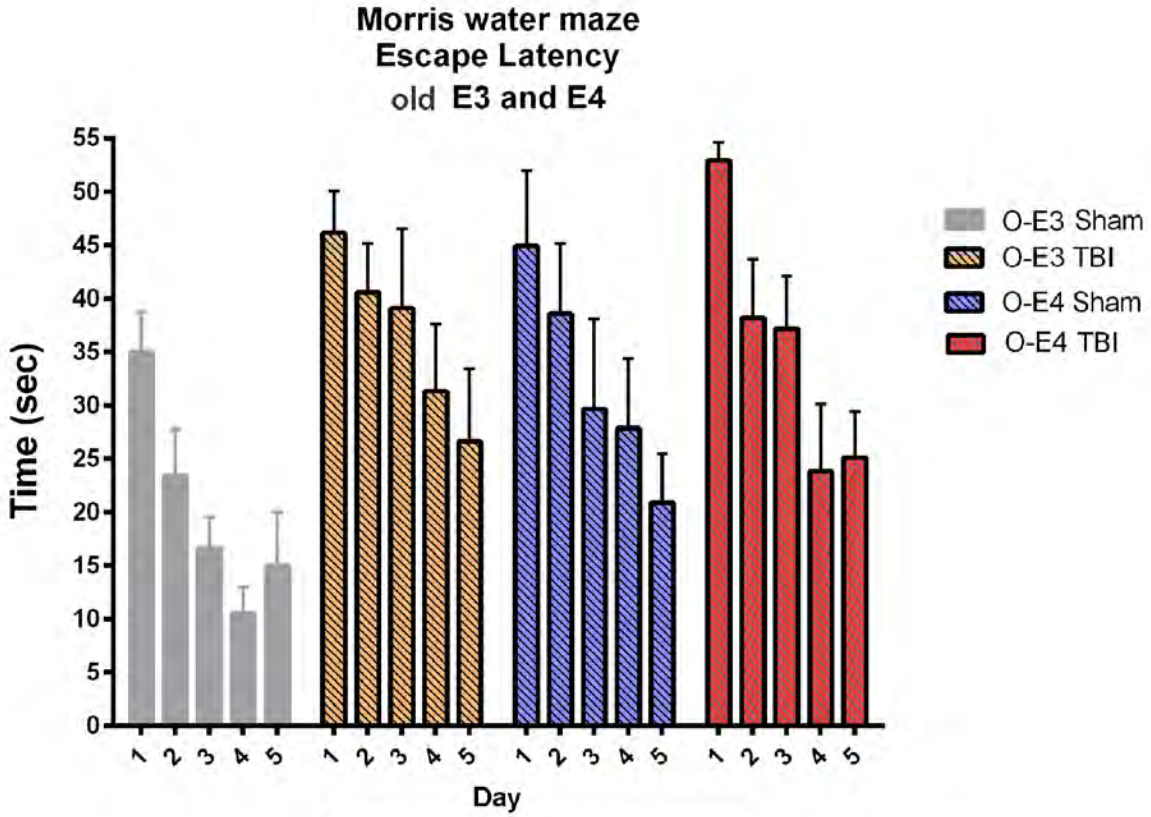


Figure 1. Changes in anxiety behavior in response to TBI (EPM data). Percent time spent in open arms is calculated based on recordings as described in the Methods and Results. Statistics by One-way Anova and Tukey post-hoc. For Old E3 and E4, (o-E3, o-E4) n=7-9 mice; y-E3 and y-E4, n=4-5.



Interaction	4.600	0.7894	ns
training	17.75	< 0.0001	****
genotype	7.595	0.0061	**

Figure 2. Young (Y) E3 and E4 mice demonstrate cognitive differences in response to TBI in MWM. Mice were trained to develop a spatial association between visual cues and a hidden platform on days 7-11 post-injury. Time (Escape latency, mean ± SEM) to find the hidden platform is shown by group for all 5 days of training. Two-way Anova (Repeated measures) was used to determine the interaction and significance/effect of major factors training/trial number and genotype. Additional explanations and comments in the text.



Interaction	1.957	0.9749	ns
training	21.33	< 0.0001	****
genotype	13.31	< 0.0001	****

Figure 3. Adult E mice demonstrate cognitive impairment differences following traumatic brain injury (TBI) in Morris water maze. Training and measurements as for young mice. Similarly to young mice, no interaction between injury/genotype and training day performance in the old mice. Performance significantly affected by genotype/injury ($p<0.0001$) and training day ($p<0.0001$).

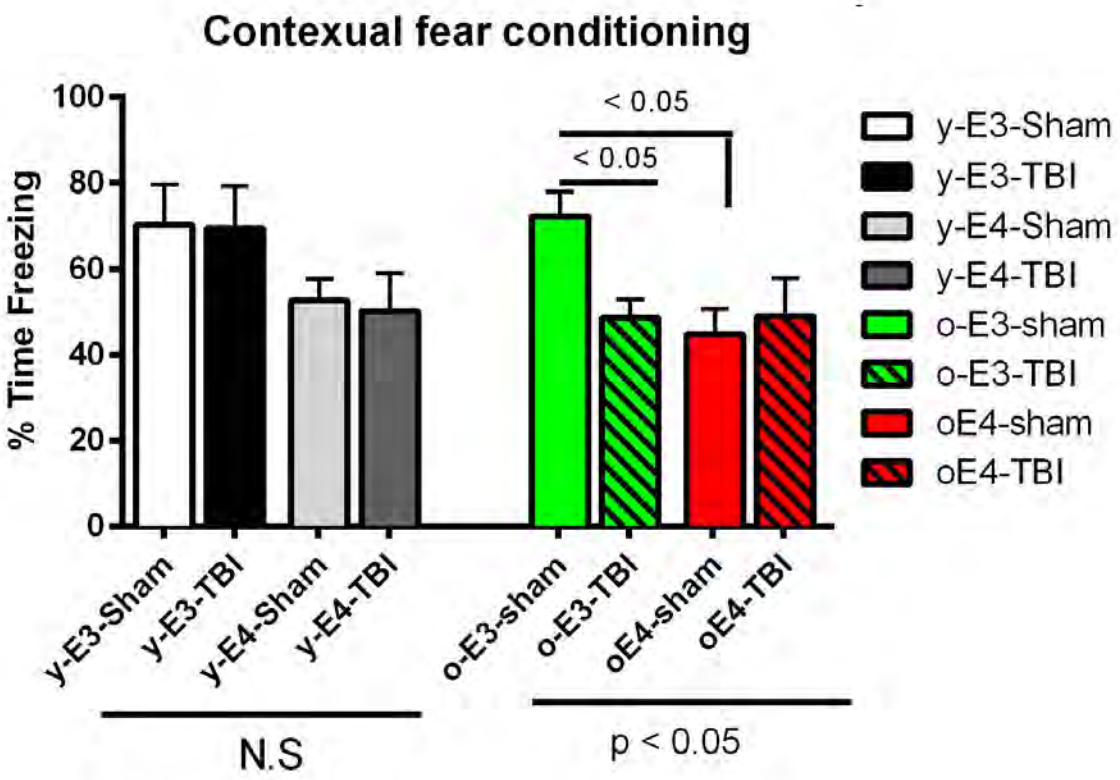


Figure 4. TBI causes age-dependent behavioral changes revealed in contextual fear conditioning paradigm. Mice were trained on days 13-14 post-injury. The graph demonstrates age-dependent effect for freezing response only in aged E3 injured vs sham mice, while neither younger E3 mice, nor E4 mice (both ages) showed difference in performance. Additional comments in the text.

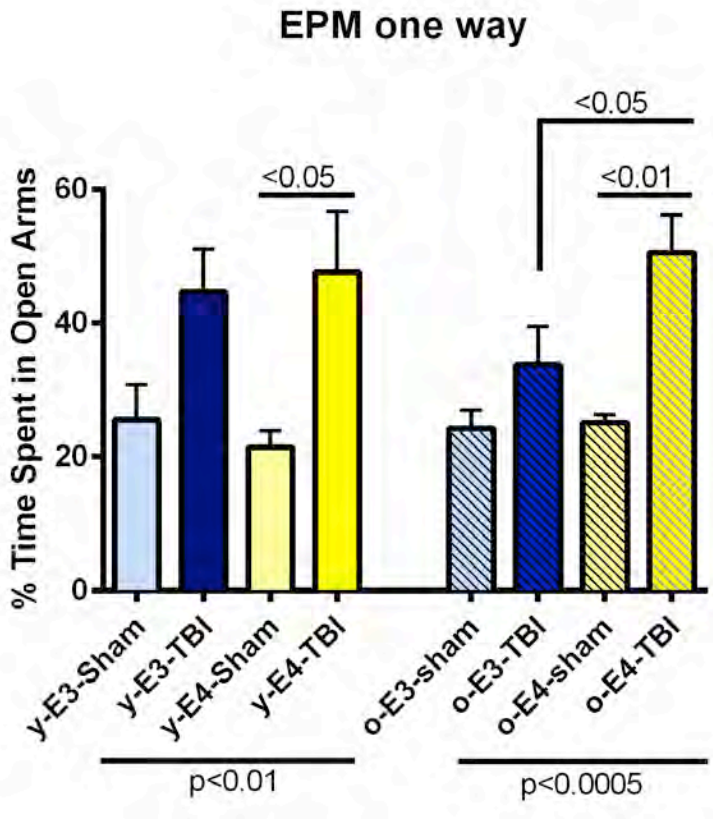
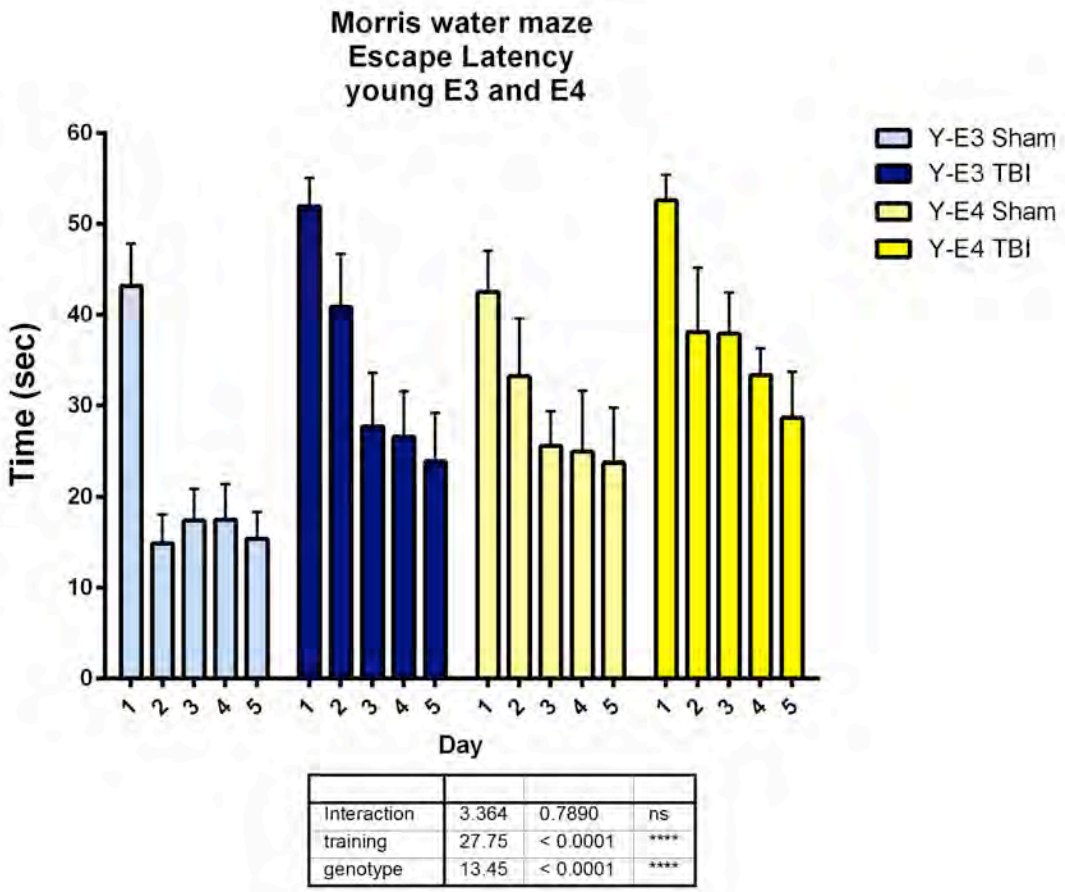
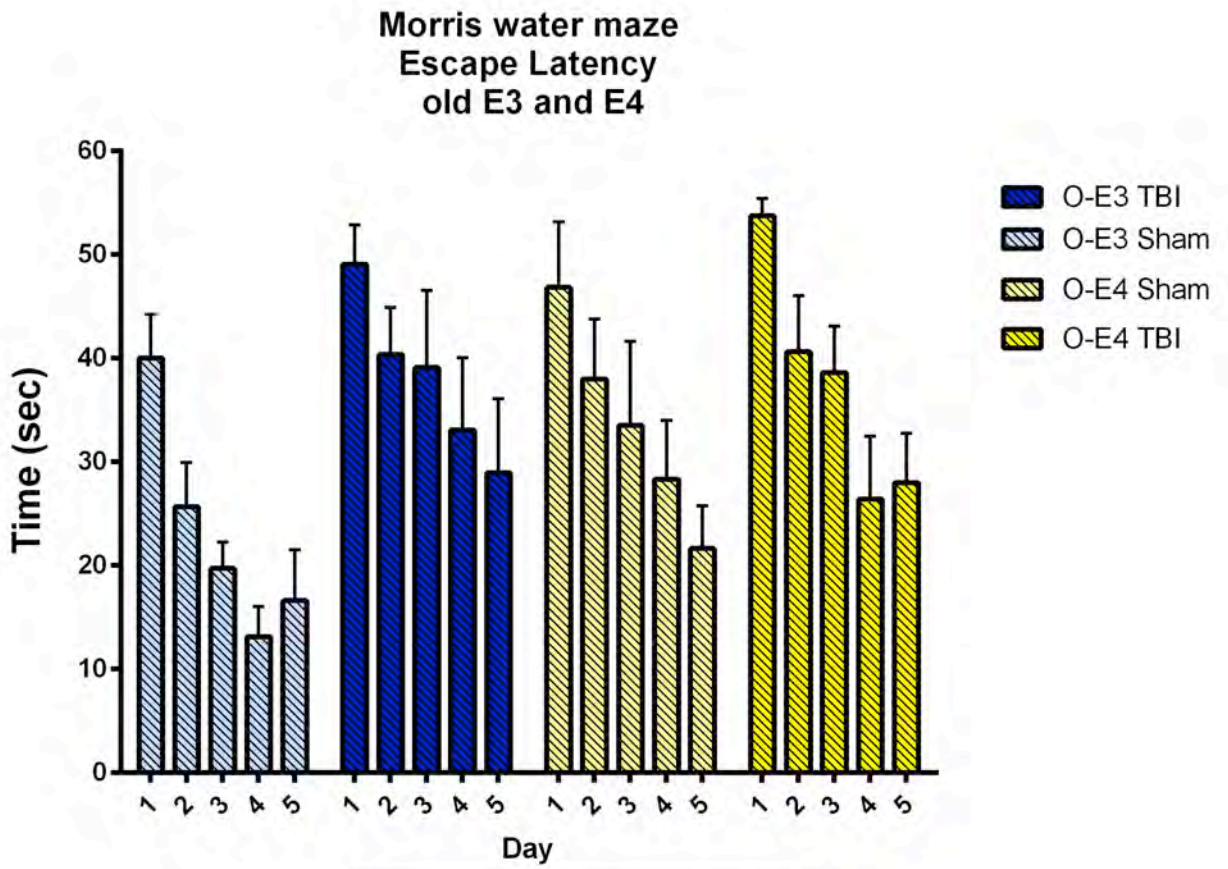


Figure 5. Traumatic brain injury (TBI) causes decreased anxiety as seen in elevated plus maze (EPM). 4DPI, we tested all mice in the EPM for anxiety-related behaviors and analyzed their behavior. TBI mice in both age groups spent significantly more time in the open arms of the maze, (young (Y), $p<0.01$; older (O), $p<0.0005$). O-E4-TBI mice spent significantly more time in open arms compared to O-E4 sham ($p<0.01$) and O-E3-TBI ($p<0.05$). In young mice, E4-TBI spent significantly more time in open arms than y-E4-sham ($p<0.05$).



Interaction	3.364	0.7890	ns
training	27.75	< 0.0001	****
genotype	13.45	< 0.0001	****

Figure 6. Following TBI, young mice demonstrate cognitive differences in response in Morris water maze. Mice were trained to develop a spatial association between visual cues and a hidden platform on days 7-11 post-injury. Time (mean + SEM) to find the hidden platform is shown by group for all 5 days of training. There was no interaction seen between injury/genotype and training day performance in the either age group, as determined by Two-way Anova, however performance was significantly affected by genotype/injury ($p<0.0001$) and training day ($p<0.00001$). All groups demonstrate learning, however sham animals learn the location of the hidden platform at a much faster rate.



Interaction	1.272	0.9957	ns
training	22.05	< 0.0001	****
genotype	11.08	< 0.0001	****

Figure 7. Following TBI, older mice demonstrate cognitive differences in response in Morris water maze. Mice were trained to develop a spatial association between visual cues and a hidden platform on days 7-11 post-injury. Time (mean + SEM) to find the hidden platform is shown by group for all 5 days of training. There was no interaction seen between injury/genotype and training day performance in the either age group, as determined by Two-way Anova, however performance was significantly affected by genotype/injury ($p<0.0001$) and training day ($p<0.00001$). All groups demonstrate learning, however E3 sham animals learn the location of the hidden platform at a much faster rate than their TBI counterparts, while there is no difference between E4 sham and TBI animals.

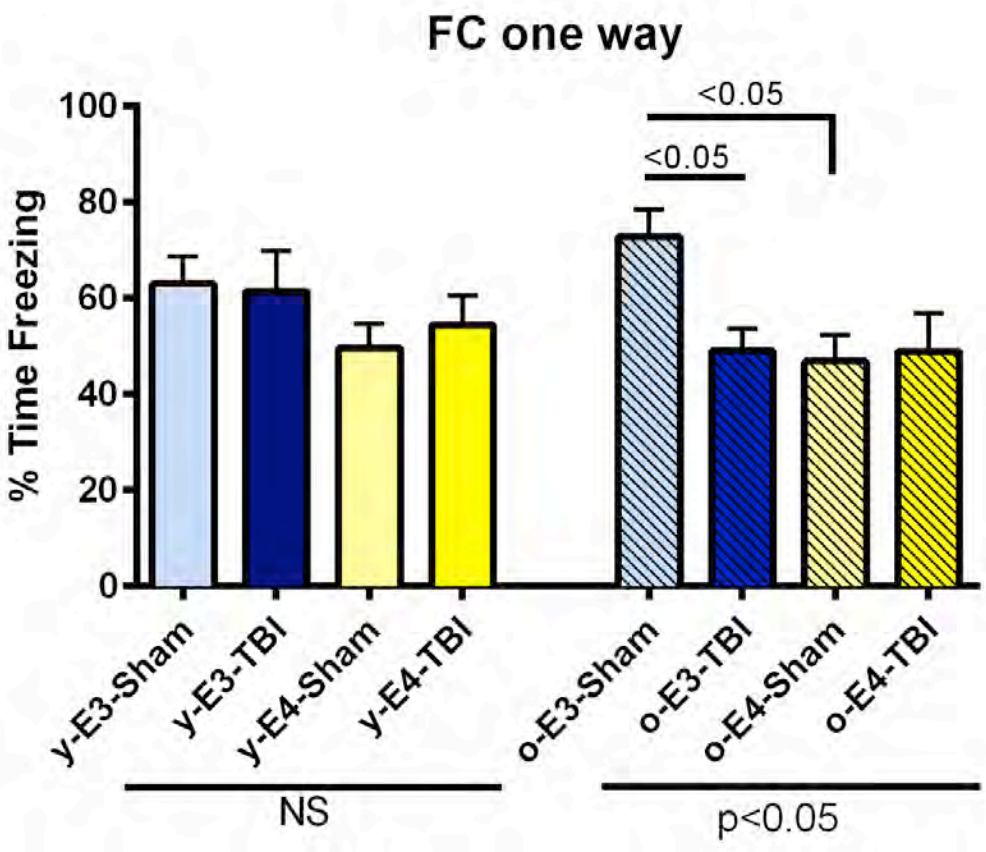


Figure 8. TBI causes age-dependent behavioral changes in Contextual Fear Conditioning. On 13 and 14DPI mice were trained with to learn an association between their environment and a foot shock, and then we tested their ability to remember this context. TBI caused an age-dependent effect in the freezing response during this paradigm, in that only the O-E3-TBI mice froze significantly more than O-E3-sham ($p<0.05$). Additionally, O-E3-sham froze for significantly more time than O-E4-sham ($p<0.05$).

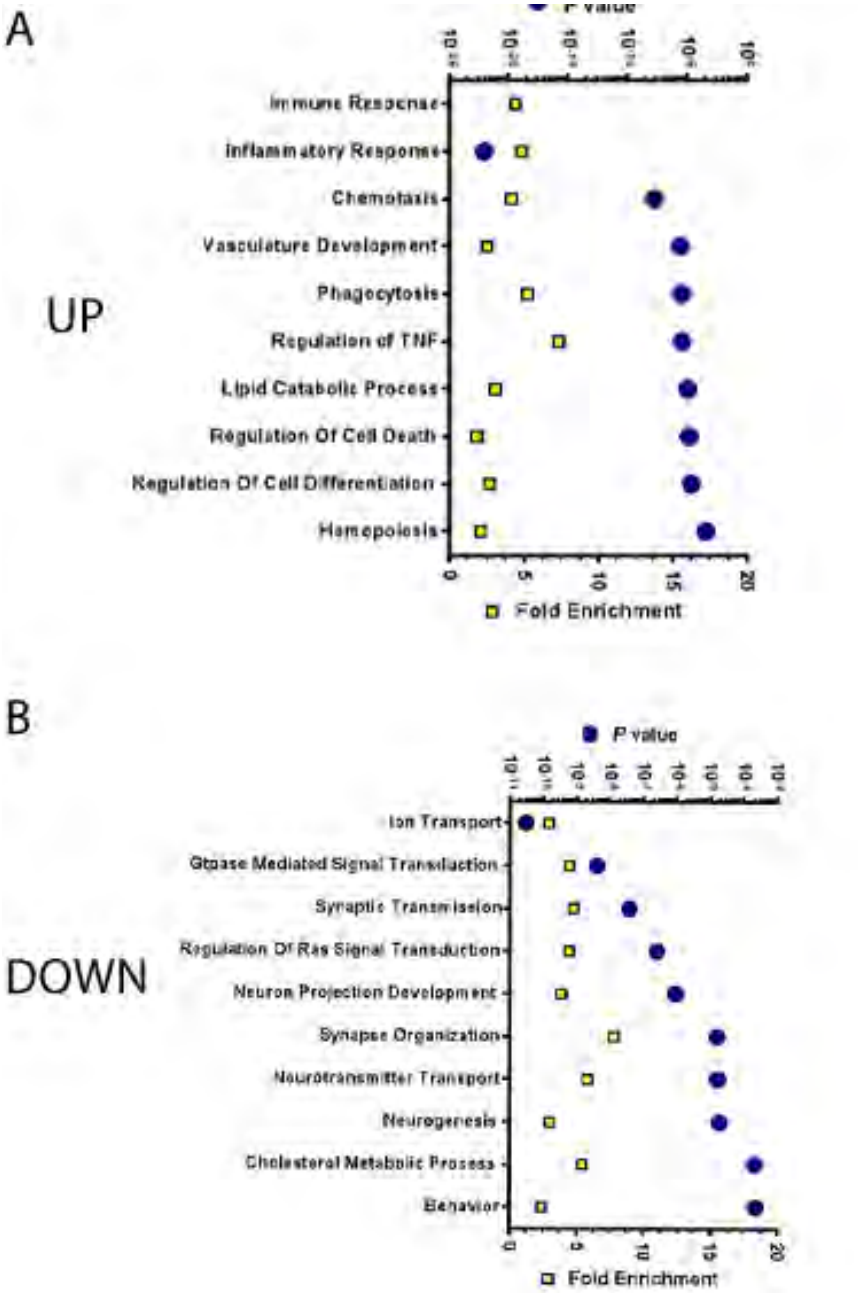


Figure 9. P-value and Fold enrichment of Common DAVID categories in Young mice. Statistically significant functional annotation categories (GOTERM Biological Process) were determined by DAVID using custom gene list generated at $p<0.05$. Highest common upregulated categories (A) show that TBI increases terms related to inflammation and immune response. Highest common downregulated categories (B) show that TBI decreases neuronal development and behavior.

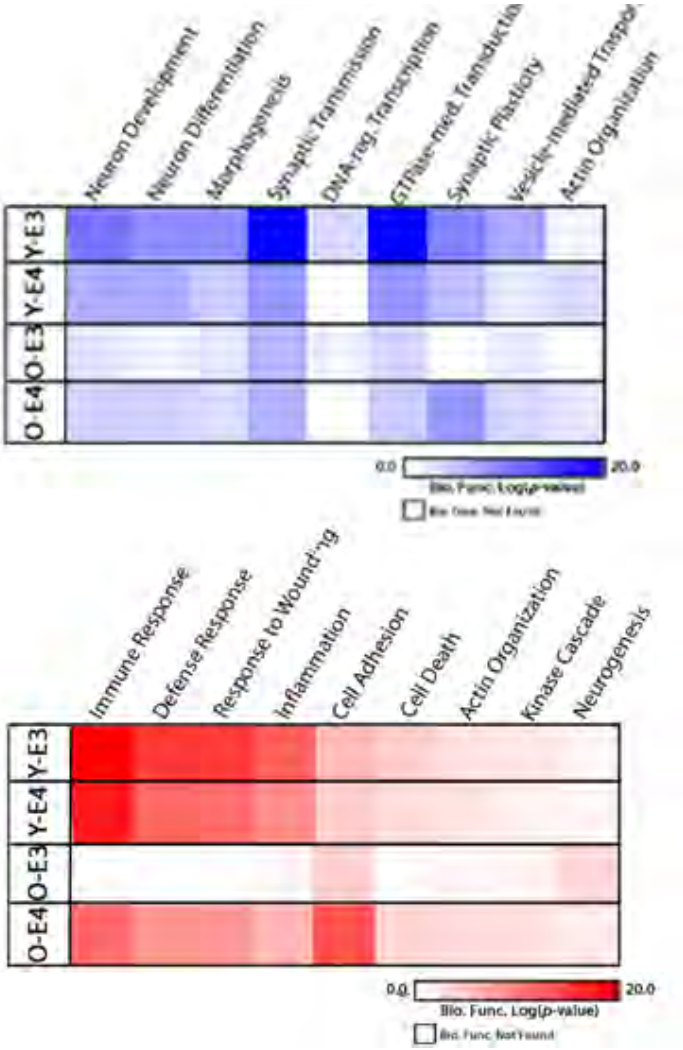


Figure 10. Heat maps of differentially expressed DAVID categories in all groups. Statistically significant functional annotation categories (GOTERM Biological Process) were determined by DAVID using differentially expressed genes at $p<0.05$. Downregulated categories are shown in panel A. Upregulated categories are shown in panel B. TBI caused an upregulation of terms related to inflammation and immune response and a downregulation of neuronal development and differentiation.

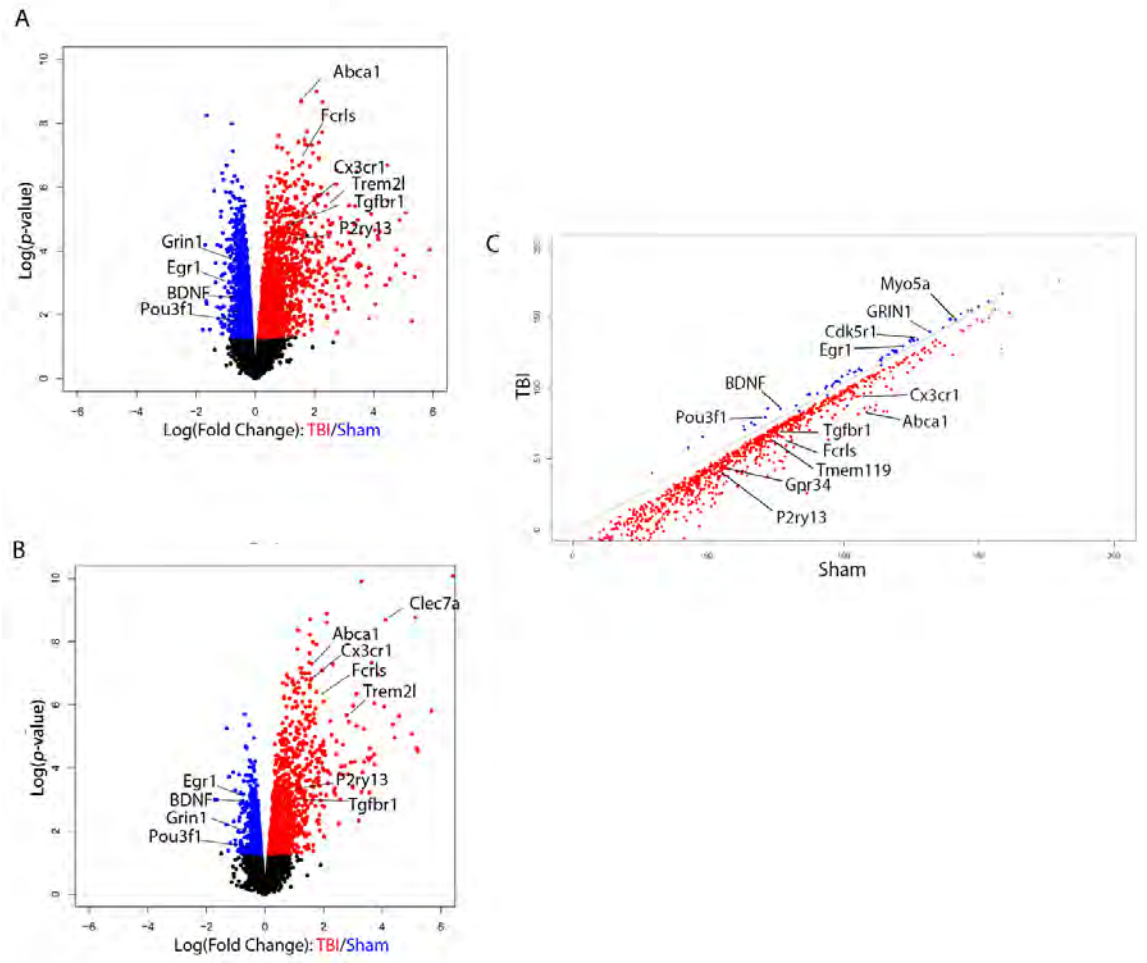


Figure 11. TBI causes genome-wide changes in gene expression. Volcano plots comparing sham and TBI gene expression in E3 mice (A) and E4 mice (B). A scatterplot (C) shows common transcriptional changes from both genotypes. Representative transcripts are shown.

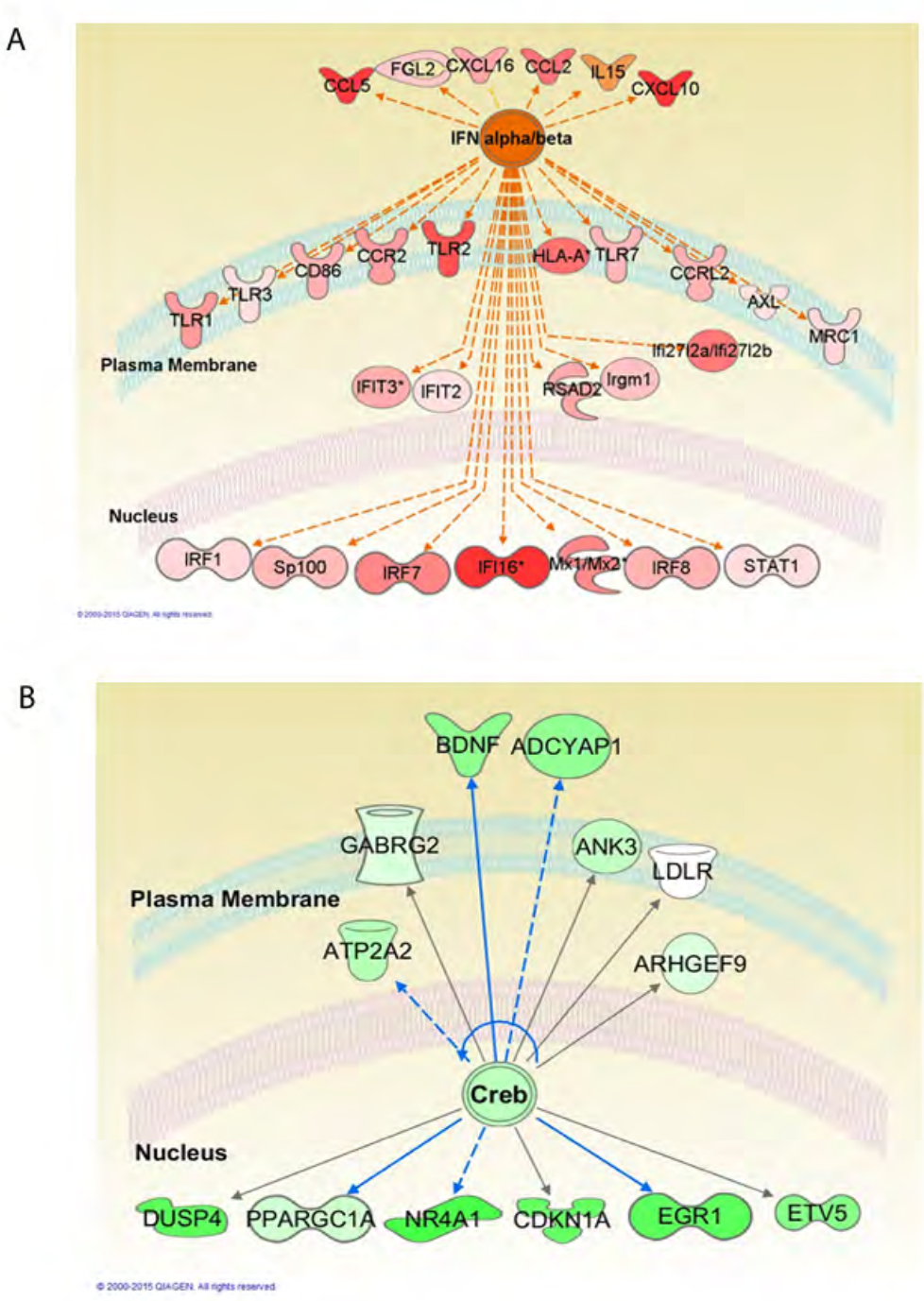


Figure 12. Pathway analysis of highest upstream effector in common gene lists. IPA analysis of the common upregulated (A) and the common downregulated (B) gene list found IFNα/β and Creb1 to be the highest common upregulated effector genes in young mice. Genes affected in this pathway found in our data are shown with gradient for highest differential expression in darker red. Genes affected in this pathway found in our results are shown with gradient for highest differential expression in darker shades, cutoff for genes was $p<0.05$.

2 Tables

Common Up-regulated			
Term	Count	P-Value	Fold Enrichment
Immune Response	106	1.20E-40	4.490486
Inflammatory Response	55	9.45E-23	4.877403
Antigen Processing And Presentation Of Peptide Antigen	20	2.78E-16	11.401720
Innate Immune Response	30	3.23E-14	5.594302
Leukocyte Mediated Immunity	27	9.94E-14	6.053160
Regulation Of Cytokine Production	28	1.01E-09	4.019311
B Cell Mediated Immunity	19	1.69E-09	5.832418
T Cell Activation	25	2.29E-09	4.300218
Chemotaxis	23	1.72E-08	4.210268
Positive Regulation Of Cytokine Production	17	4.11E-08	5.470987
Prostaglandin Metabolic Process	8	1.92E-06	11.401720
Prostanoid Metabolic Process	8	1.92E-06	11.401720
Vasculature Development	32	2.80E-06	2.553985
Phagocytosis	13	3.74E-06	5.293656
Regulation Of Tumor Necrosis Factor Production	10	3.88E-06	7.390004
Common Down-regulated			
Term	Count	P-Value	Fold Enrichment
Ion Transport	49	3.14E-11	2.922279
Regulation Of Small Gtpase Mediated Signal Transduction	24	4.11E-09	4.469737
Transmission Of Nerve Impulse	23	1.74E-08	4.321405
Synaptic Transmission	20	3.83E-08	4.771067
Regulation Of Ras Protein Signal Transduction	19	2.59E-07	4.457390
Neuron Projection Development	20	9.31E-07	3.895642
Exocytosis	14	1.77E-06	5.404318
Synapse Organization	9	1.65E-05	7.799235
Neurotransmitter Transport	11	1.70E-05	5.838594
Neuron Development	21	1.90E-05	3.053810
Neuron Differentiation	25	2.46E-05	2.660558
Cholesterol Biosynthetic Process	6	1.64E-04	11.077174
Cholesterol Metabolic Process	9	2.25E-04	5.459464
Behavior	23	2.35E-04	2.411451
Steroid Metabolic Process	13	4.18E-04	3.428649
Insulin Secretion	5	0.003414486	7.863426

Table 1. List of up- and down-regulated functional annotation categories in young mice following TBI. Statistically significant functional annotation categories (GO TERM Biological Process) were determined using DAVID and a custom gene list generated at $p < 0.05$ as in the edgeR output Differential Expression (DE) Tables. TBI causes up-regulation of functional annotation categories related to immune system and inflammation in young adult mice

regardless of genotype, whereas a decrease was seen in categories related to neuronal structure and metabolism. N = at least 4 libraries per genotype / condition

E3 only: Up-regulated			
Term	Count	P-Value	Fold Enrichment
Translation	27	1.03E-04	2.328100
Response To Steroid Hormone Stimulus	9	0.001381475	4.125911
Protein Transport	40	0.00143566	1.690081
Response To Hypoxia	9	0.002106544	3.868041
Response To Oxygen Levels	9	0.002327705	3.808533
Response To Glucocorticoid Stimulus	5	0.00429852	7.238440
Regulation Of Transcription Factor Activity	8	0.005293878	3.729637
Negative Regulation Of Transcription Factor Activity	5	0.007444608	6.251380
Positive Regulation Of Transport	12	0.008580202	2.500552
Actin Cytoskeleton Organization	13	0.017147867	2.167145
E3 only: Down-regulated			
Term	Count	P-Value	Fold Enrichment
Neuron Projection Development	30	2.32E-06	2.678952
Neuron Development	36	2.53E-06	2.400047
Regulation Of Synaptic Plasticity	13	3.82E-06	5.272326
Cell Projection Morphogenesis	28	4.70E-06	2.698403
Homophilic Cell Adhesion	20	7.36E-06	3.327701
Visual Learning	10	9.25E-06	6.712775
Protein Localization	67	1.22E-05	1.732128
Vesicle-Mediated Transport	47	1.51E-05	1.963415
Regulation Of Neuron Differentiation	17	5.91E-05	3.244508
Regulation Of Neurological System Process	18	6.04E-05	3.100946
Neuron Differentiation	40	8.33E-05	1.951584
Positive Regulation Of Transcription, DNA-Dependent	40	2.01E-04	1.871832
Positive Regulation Of RNA Metabolic Process	40	2.33E-04	1.858429
Protein Kinase Cascade	23	0.005007425	1.897212

Table 2. List of up- and down-regulated functional annotation categories in young E3 mice. Statistically significant functional annotation categories BP were determined using DAVID and a custom gene list generated at $p < 0.05$ as in the edgeR output DE Tables. In addition to the common categories related to immune and inflammatory response (presented on Table 1), TBI caused additional up-regulation in BP Terms related to respiration, molecular transport and transcription factor activity. Additional functional annotation categories unique to E3 mice and related to synaptic plasticity and differentiation were down-regulated. N = at least 4 libraries per genotype / condition

E4 only: Up-regulated			
Term	Count	P-Value	Fold Enrichment
Cell Adhesion	28	1.47E-04	2.230885
Regulation Of Cell Death	26	8.43E-04	2.064177
Cell Migration	15	0.001001	2.793586
Respiratory System Development	10	0.001828	3.604626
Extracellular Matrix Organization	9	0.001872	3.982934
Protein Kinase Cascade	14	0.002493	2.651539
Cytoskeleton Organization	17	0.002681	2.330844
E4 only: Down-regulated			
Term	Count	P-Value	Fold Enrichment
Cytoskeleton Organization	14	0.001132	2.888781
Actin Filament-Based Process	10	0.001228	3.822007
Cellular Component Morphogenesis	14	0.002202	2.683027
Response To Hypoxia	6	0.002534	6.306312
Response To Oxygen Levels	6	0.002712	6.209292
Negative Regulation Of Map Kinase Activity	4	0.003019	13.453465
Cholesterol Biosynthetic Process	4	0.004540	11.698666
Regulation Of Cell Cycle	10	0.004595	3.143333
Regulation Of Transmission Of Nerve Impulse	7	0.005104	4.400666
Cellular Ion Homeostasis	11	0.005523	2.835021
Lipid Biosynthetic Process	11	0.009976	2.596283

Table 3. List of up- and down-regulated functional annotation categories in young E4 mice. Statistically significant functional annotation categories BP were determined using DAVID and a custom gene list generated at $p < 0.05$ as in the edgeR output DE Tables. In addition to the common categories related to immune and inflammatory response (presented on Table 1), TBI caused additional up-regulation in BP Terms cell movement and localization, as well as cell death. Additionally, functional annotation categories cell structure and organization, as well as response to oxygen levels and hypoxia were uniquely down-regulated in injured E4 mice.